Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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Sixty Years of Mystery

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NO induced mutations fascinated H. J. MULLER more than the five *white-mottled* strains (w^{m1}-w^{m5}) recovered among the progeny of X-ray-treated flies (MULLER 1930). Radiation treatment was providing unprecedented opportunities to systematically mutate genes and rearrange chromosomes in *Drosophila melanogaster*. Most induced mutations behaved like previously studied spontaneous lesions. In contrast, the variegated strains displayed novel and perplexing properties. Each individual from these strains showed variable expression of the *white* gene among the hundreds of ommatidia in the compound eyes. This suggested that the gene was "eversporting," *i.e.*, that it underwent frequent genetic changes during eye development like previously described mutable genes in maize and *Drosophila virilis*. However, unlike mutable genes, germinal changes in the *white-mottled* strains occurred very rarely, no reversions to a stable state appearing after more than 50 generations (MULLER 1930).

Several additional properties distinguished the lesions in the eversporting strains from simple gene mutations. Linkage analysis showed that all the mottled strains had undergone chromosome rearrangements. Furthermore, the w^{m1} strain also showed variable expression of *Notch*, a gene separated from *white* by 1.5 map units. Effects on the two genes were coupled in some way because flies with strong *white* mottling usually had notched wings while weakly mottled flies did not. Existing theory seemed inadequate to explain these "peculiar manoeuvers of some portion of chromatin larger than a gene which has been displaced from its original position" (MULLER 1930).

MULLER's "eversporting displacements" displayed an unusual susceptibility to modification. The w^{m1} strain immediately gave rise to strongly and weakly mottled derivatives. As reported in GENETICS (GOWEN and GAY 1934), addition of a *Y* chromosome was shown to suppress variegating rearrangements and removal of the *Y* to enhance them. Presumably, MULLER's dark-eyed w^{m1} lines contained an extra *Y* chromosome. Subsequently, it was shown that altering the dosage of heterochromatin on other chromosomes also modified variegation. Temperature altered mottling in an unexpected direction: unlike most familiar chemical reactions, variegation was increased at low temperature. However, the discovery that variegation could be predictably modified only increased the mystery surrounding the mechanisms that produced such lesions.

JACK SCHULTZ, one of MORGAN's last graduate students at Columbia, maintained an interest in variegation over much of his career. He was one of the first to apply the new technique of polytene chromosome analysis to eversporting strains. SCHULTZ (1936) found that rearrangements producing variegated effects always involved breakage within heterochromatic ("inert") regions. The unusual effects on gene expression therefore appeared to result from placing genes abnormally close to the centric heterochromatic regions. Furthermore, phenotypic instability was paralleled by variation in the banding pattern near the rearrangement breakpoint among individual salivary gland cells (CASPERSSON and SCHULTZ 1938). MILISLAV DEMEREC (1941) carefully studied rearrangements involving genes in the *Notch* region and established that both phenotypic and cytogenetic variegation could affect multiple genes and polytene chromosome bands, but in a polar manner that always seemed to "spread" from the heterochromatin.

In 1937, SCHULTZ described the appearance of a third-chromosome genetic modifier that "exercises a

dominant maternal effect for the suppression of variegation" (MORGAN, BRIDGES and SCHULTZ 1937). It soon became apparent that variegating rearrangements were sensitive to changes in genomic elements besides the major heterochromatic blocks. In an attempt at a more systematic study of background influences, SCHULTZ induced a large number of dominant suppressors (*Suvar*) and enhancers (*Evar*) of w^{m4} variegation (MORGAN, SCHULTZ and CURRY 1941). This collection included an unusual group of autosomal modifiers "deficient for regions within the sections ordinarily called euchromatic." SCHULTZ believed that these modifiers corresponded to deletions of "interstitial" heterochromatin and could be used to map and study such domains (MORGAN and SCHULTZ 1942). The realization that variegation could be influenced in *trans* by the activity of "normal" euchromatic genes dawned slowly. SPOFFORD (1967) used recombination to map precisely the first *Suvar* to a specific locus on *2L*.

Direct proof that variegation was caused by a position effect required that the gene be separated intact from its association with heterochromatin. Several investigators showed that variegation could be alleviated by secondary rearrangements, but the reinversion study of *In(1)roughest*^{3} reported by ED NOVITSKI (1961) was probably the most elegant. However, the possibility remained that these reversions might have resulted from secondary mutations, and many of the so-called reversions could be demonstrated to still variegate under appropriate conditions. The Russians N. B. DUBININ and B. N. SIDOROV (1935) and I. B. PANSHIN (1935) reported beautiful experiments in which variegating genes were cleanly separated from the inducing rearrangement by recombination. The "variegating" allele then behaved like a normal, wild-type gene when present on an unrearranged chromosome; conversely, a wild-type allele from a normal-sequence chromosome variegated when recombined onto the rearranged homolog. These studies also eliminated a role for structural heterozygosity between homologs, a component of some early models. Final acceptance of these proofs followed analogous studies on a variegating *white* rearrangement by BURKE JUDD (1955).

Forays into understanding the relationship between development and variegation began as early as 1930 with MULLER who, aided by THEODOSIUS DOBZHANSKY's dissecting skills, discovered that the original w^m rearrangements were associated with mottled pigmentation of the testis sheath as well as in the eye. More detailed studies revealed that, for a given rearrangement, different larval and adult tissues and even bristle cells in different regions of the notum (NOUJDIN 1936) could vary widely in the probability of expressing the variegated phenotype and in the type of variegation (fine grain or large patches). The idea that expression of the variegated phenotype was influenced by events occurring many cell divisions prior to final action of the gene was hinted at by studies of S. Y. CHEN (1948), SCHULTZ (1956) and others who determined that *white* mottling could be altered by temperature shifts applied not only during the time of eye differentiation (pupariation) but also early in development (blastoderm to hatching). These temperature-sensitive periods were later confirmed by correlating the size of variegating patches with the size of marked clones induced at specific times in development. Surprisingly, variegation could even be modified prior to fertilization. The parental source of the rearrangement and the genetic constitution (*i.e.*, presence or absence of a *Y* chromosome) of the parent were found to greatly influence the extent of *In(1)scute*^{8} (sc^8) variegation in the progeny both phenotypically (NOUJDIN 1944) and cytogenetically (PROKOFYEVA-BELGOVSKAYA 1947).

As with most genetic phenomena, interesting exceptions appeared almost immediately that complicated the "rules" established by the properties of most variegating rearrangements. MULLER (1930) reported the appearance of rearrangements that displayed a dominant eye color variegation (*Plum*, later called *brown-dominant* or bw^V). Most other variegating genes behaved as recessives in the presence of a wild-type allele on the normal sequence homolog. Although bw^V rearrangements displayed many similarities to recessive variegation (association with heterochromatic breakpoints, *Y* suppressibility), attempts to force *bw* into the prevailing view of variegation were generally unsuccessful. Another exception was "reverse" position effects exhibited by normally heterochromatic genes such as *light* (*lt*) (SCHULTZ and DOBZHANSKY 1934), *cubitus interruptus* (*ci*) (DUBININ and SIDOROV 1934) and, in *Drosophila virilis*, *peach* (BAKER 1953). Variegation occurred when these heterochromatic genes were moved to distal euchromatin (HESSLER 1958); *lt* variegation was enhanced (instead of suppressed) by an extra *Y* chromosome.

By the end of the 1930s the phenomenology of position-effect variegation was clear. It was less certain whether heterochromatic rearrangements in organisms other than Drosophila produced variegation. DAVID CATCHESIDE's (1939) discovery of a rearrangement that exerted a position effect on the *P* locus in *Oenothera blandina* supported the view that these effects were more widespread. However, the underlying mechanisms responsible for position-effect variegation remained obscure. DEMEREC (1941) summarized the possible explanations in terms that have hardly changed: "This instability may be due either to a reversible chemical change in the gene or may be caused by a reversible suppression of the activity of

the gene." Arguments between these two views comprise much of the subsequent history of research on this subject.

SCHULTZ's observations of variegating rearrangements in polytene chromosomes convinced him that genic material had been lost, possibly due to a change in replication within those cells whose expression was weakened (MORGAN, BRIDGES and SCHULTZ 1936). SCHULTZ took ultraviolet pictures of variegating chromosomes in TORBJÖRN CASPERSSON's laboratory in Stockholm and thought he had detected at least one example where the absorbance of a band had changed, although it showed an increase instead of the expected l ;s (CASPERSSON and SCHULTZ 1938). However, P. A. COLE and E. SUTTON (1941) were unable to confirm the findings.

The alternative view, that variegation suppressed gene activity, was bolstered by other cytogenetic studies. A. A. PROKOFYEVA-BELGOVSKAYA (1939) noted striking variation between individual salivary gland cells in the appearance of the polytene chromosome regions that contained variegating genes. Whereas SCHULTZ thought the affected regions had been lost in some cells, she interpreted these changes as a transformation of the bands to a heterochromatic state. The cytological analysis of heterochromatin was inherently subjective; these different interpretations were never resolved. The difficulties were underscored by PROKOFYEVA-BELGOVSKAYA's 1937 GENETICS publication describing the detailed structure of the *Y* chromosome in salivary gland cells, but other cytologists have been unable to detect this chromosome.

The many similarities between position-effect variegation and mutable genes in maize led BARBARA MCCLINTOCK (1950, 1951) to postulate a close relationship between the two phenomena. Like Drosophila variegation, maize controlling elements were linked to heterochromatin because cytogenetic and irradiation studies suggested that they derived from rearranged heterochromatin regions. The suppression of *Ds*-induced mottling by an extra dose of *Ac* appeared similar to the suppression of variegation by an extra *Y* chromosome. This view generated little interest among workers studying position-effect variegation.

The volume of literature devoted to position effects decreased substantially between 1950 and the late 1970s despite a seminal review by E. B. LEWIS (1950) that introduced the problem to a new generation of students. Nevertheless, each new fact learned about heterochromatin stimulated new approaches to understanding the mechanism of variegation. The discovery that heterochromatin replicated late in the cell cycle led J. HEBERT TAYLOR (1964) to resurrect a relationship between replication and position-effect variegation. The finding that the copy number of DNA sequences within heterochromatic regions was drastically reduced within polytene salivary gland cells (GALL, COHEN and POLAN 1971; HENNIG and MEER 1971) also suggested that changes in replication might lead to underrepresentation of variegating genes. WARGENT and HARTMANN-GOLDSTEIN (1974) found that incorporation of thymidine into a variegating region was not synchronized with its normal counterpart. Late replication and slightly reduced DNA content were detected in some salivary gland cells within a variegating region under enhanced conditions (ANANIEV and GVOZDEV 1974). The limited copy-number changes observed in these and subsequent studies (HENIKOFF 1981; KORNHER and KAUFMAN 1986) called into question whether changed DNA content was relevant to variegated gene expression.

Finally, molecular studies using cloned genes revealed that both of DEMEREC's proposed mechanisms, gene inactivation and gene loss, could be produced by juxtaposition with heterochromatin. The copy number of the *rosy* gene within the variegating rearrangement $ry^{ps11187}$ was shown to remain unchanged despite a sevenfold reduction in enzyme activity (RUSHLOW, BENDER and CHOVNICK 1984). In this case, position-effect variegation affects gene activity rather than copy number. However, studies of the sc^{8} junction in the minichromosome *Dp(1;f)1187* yielded a different result (KARPEN and SPRADLING 1990). Euchromatic sequences spanning more than 100 kb adjacent to the breakpoint were underrepresented as much as 39-fold in larval salivary glands. The degree of underrepresentation varied among individual cells and was suppressed by a *Y* chromosome. Reduced gene copy number might therefore be sufficient to explain the *yellow* variegation associated with this rearrangement, although an independent effect on transcription could not be ruled out.

Because many different heterochromatic breaks can induce mottling, inducing sequences must be dispersed within the heterochromatin of all the chromosomes. Several factors have complicated searches for specific inducing sequences. In particular, the quantity as well as the quality of specific *cis*-regulatory sequences may influence the nature of induced variegation. For example, retrotransposons were found at the breakpoints of three inversions variegating for *white*, including w^{m4} (TARTOF, HOBBS and JONES 1984). X-ray-induced revertants were obtained that still retained the transposon sequences, suggesting that variegation was induced by sequences located within heterochromatin some distance from the breakpoint. However, the apparent revertants of w^{m4} still exhibited mottling in the presence of strong variegation enhancers, indicating that the strength of variegation was related to the quantity of heterochro-

matin adjacent to the breakpoint (REUTER, WOLFF and FRIEDE 1985).

Some recent progress in our understanding of the molecular nature of *cis* sequences involved in variegation has been generated by studies of the "exceptional" variegating systems. The molecular cloning of the *bw* locus (HENIKOFF and DREESEN 1989) and a deletion analysis of a rearrangement that causes variegation of a transformed copy of *brown* suggested that sequences close to or within the gene, as well as somatic pairing between homologues, are essential components of *bw* dominant variegation (HENIKOFF 1990). The *lt* gene is embedded in a region rich in repetitive sequences (DEVLIN, BINGHAM and WAKIMOTO 1990). This unusual organization may underlie the reverse position effects observed in lt^V rearrangements. Recently, genes inserted into telomeric heterochromatin via *P* element transformation have been observed to undergo *Y*-suppressible position effects (G. KARPEN and A. M. SPRADLING, unpublished observations). Variegation-inducing sequences may be easier to localize within the relatively small blocks of heterochromatin at telomeres.

The proteins encoded by some modifier loci have been characterized recently. TOM GRIGLIATTI, GÜNTER REUTER and KEN TARTOF have systematically screened for loci that dominantly enhance or suppress w^{m4} variegation (see SINCLAIR, MOTTUS and GRIGLIATTI 1983; REUTER *et al.* 1990; LOCKE, KOTARSKI and TARTOF 1988). These studies have identified many new loci with strong effects on variegation and suggest that at least 50 such loci exist within the Drosophila genome. Variegation is sensitive to the dosage of many of these loci. Most commonly, a single dose suppresses variegation while three doses enhance; however, the reverse situation holds for a few other genes. Some of the genes mutate to lethality or female sterility, suggesting that they play a role in development. REUTER *et al.* (1990) recently cloned *Suvar(3)7* and showed that it encodes a putative DNA-binding protein containing several predicted zinc fingers. Another locus, *Suvar(2)5*, may encode the C1A9 heterochromatin-binding protein (see EISSENBERG 1989). Further study of modifier loci should provide a wealth of important information on variegation, heterochromatin, and the developmental control of chromosomal functions.

Although we have learned that variegating rearrangements can suppress gene activity and sometimes are associated with sequence underrepresentation, the task remains of elucidating the molecular mechanisms that produce these changes. A detailed heterochromatinization model has been formulated (ZUCKERKANDL 1974; SINCLAIR, MOTTUS and GRIGLIATTI 1983; LOCKE, KOTARSKI and TARTOF 1988). Chromosome compaction was postulated to initiate at specific sequences within centric DNA and spread until termination sequences were encountered, due to the formation of a large complex of proteins. Rearrangements placing euchromatic sequences within such a domain would become partially heterochromatinized as the protein complex assembled along its length. Enhancer and suppressor loci were postulated to encode structural proteins of the complex or modifiers of such proteins. Presumably, the *Y* chromosome suppresses variegation by titrating these heterochromatin-binding proteins, reducing the tendency of heterochromatin to spread across the breakpoint.

Recent progress in understanding variegation at the molecular level has encouraged some workers to conclude that the heterochromatization model is essentially correct and that position-effect variegation can now join the mainstream of molecular biology (EISSENBERG 1989). Unfortunately, such optimism seems premature. Several properties of variegation are not simply explained by spreading domains of heterochromatin. Why should a heterochromatin complex move a variable distance along euchromatin in different cells, but after some point in development faithfully maintain its position through multiple cell cycles? How can parental source effects be explained by maternal heterochromatin titrating binding proteins during oogenesis, when heterochromatin is severely underrepresented in nurse cells? Critical tests of the model will be difficult to accomplish. For example, even if modifier loci are shown to encode proteins that bind heterochromatic DNA, it would be necessary to show that such proteins actually mediate heterochromatin formation in a manner that can spread along a chromosome region.

There are alternatives to spreading domains of heterochromatin. The strong similarities between position-effect variegation and mutable genes were noted by MULLER, but the germinal stability of genes affected by variegating rearrangements differentiated these two phenomena. This objection carries little weight since the discovery that transposable elements can be subject to different regulation in somatic and germline cells. KARPEN and SPRADLING (1990) suggested that transposable elements located within heterochromatin might induce position-effect variegation in somatic cells by transposing into juxtaposed euchromatin. The relevant mobile elements would have to transpose predominantly locally and conservatively to explain the spreading effect; multiple rounds of cell division may be required to invade hundreds of kilobases of DNA. Transposons are known to suppress the transcription of genes near their site of residence and this suppression is subject to modification by genomic suppressor loci (SPANA, HARRISON and CORCES 1988). Furthermore, transposons can catalyze imprecise excisions that would lead to the ob-

served reductions in sequence copy number. Transposons encode regulators of their own activity; the modifying effects of added heterochromatin would result from altering the dosage of heterochromatic transposons and their genes. Transposon regulatory proteins could be inherited maternally to account for parental source effects. In this model it is easy to understand why gene inactivation would be stochastic but relatively stable, because a covalent change (an inserted element) would be responsible.

Position-effect variegation has now fascinated several successive generations of geneticists. This phenomenon should remind us that very basic aspects of chromosome structure and function remain poorly understood. Although 60 years of research have still not solved the problem, systems have been developed that should facilitate an understanding of position-effect variegation at the molecular level. The continued interest of geneticists speaks to the striking nature of the phenomenon and suggests that, when the solution does emerge, it will have been worth waiting for.

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